CURRENT STATUS OF BIOHERBICIDE DEVELOPMENT AND PROSPECTS FOR RICE IN ASIA

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ABSTRACT

Biological control of weeds is the deliberate use of natural enemies to suppress the growth or reduce the population of a problem weed species. Augmentation of indigenous fungal plant pathogens (bioherbicides/mycoherbicides) is a biocontrol strategy which involves the application of living inoculum of an endemic plant pathogen, generally a fungus, in a manner very similar to that of a chemical herbicide. Bioherbicides are applied in an attempt to overcome disease restraints by periodically dispersing an abundant supply of virulent inoculum onto a susceptible weed population. The application is timed to take advantage of favorable environmental conditions and/or the most susceptible stage of weed growth. Low virulence of the pathogen and fastidious environmental conditions are the two major biological restraints: formulation and scale-up "fermentation" are the primary technological restraints: and depending on market opportunities, economic and regulatory aspects are also potential restraints to bioherbicide development. Bioherbicide research directed towards tropical weeds has only recently commenced with active programs in the Republic of Korea, Japan, Mainland China and the Philippines. Biological control is usually limited to one or a few closely related species and as such cannot presently be considered as an alternative to broad spectrum chemical herbicides and other weed control tactics, but should be considered as a complementary strategy in integrated weed management systems.

INTRODUCTION

Weeds are a major problem in all Southeast Asia rice production systems including irrigated, rainfed, upland, and deep water rice. Weeds in rice commonly cause yield losses of 10 to 40% and occasionally losses of 100%. Unlike the periodic and often dynamic outbreaks of diseases and insects pests, weeds are a constant pest component. Available weed control strategies include water and other management practices, hand weeding, mechanical, chemical herbicides, and biological control. Each method has advantages and limitations (Watson 1992).

Managing water for weed control can provide good control of some weeds in irrigated lowlands but other species are tolerant. Hand weeding is expensive, time consuming, and particularly difficult for spiny, poisonous, and perennial weed species. Mechanical weeders are effective in most transplanted rice but are ineffective in direct seeded systems. Herbicides can provide effective weed control in many rice systems, but costs, availability, and concerns associated with widespread and increased use, bring into question the continued reliance on chemical herbicides in rice systems in Asia. Applicator safety, environmental contamination, and population shifts towards more noxious troublesome weeds are some of the concerns expressed. The use of insects or pathogens as biocontrol agents of rice weeds has received limited study.

Keywords: biological control, control efficacy, indigenous fungi.

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There is an urgent need: 1) to discover and develop new weed control technologies and 2) to improve existing weed control technologies that are economically and environmentally sustainable. Biological weed control, primarily the augmentation of indigenous fungal plant pathogens or the inundative approach, has great potential to reduce chemical inputs and to provide viable, economic, effective weed control components within IPM programs in rice.

BIOLOGICAL WEED CONTROL

Biological weed control is the deliberate use of natural enemies to suppress the growth or reduce the population of a weed species. It involves two primary strategies, the classical or inoculative strategy and the inundative or bioherbicide (mycoherbicide) strategy. Numerous comprehensive reviews describe in detail the fundamentals, methodologies, and progress of biological weed control (Charudattan 1991, Hasan and Ayers 1990, Julien 1992, Schroeder 1983, TeBeest and Templeton 1985, Wapshere 1982, Watson 1989, Watson 1991b).

The use of the terms "bioherbicide" and "mycoherbicide" is unfortunate as it often conjures up images of chemical pesticides and the involvement of large industries. The approach should more appropriately be described as: the augmentation of indigenous fungal plant pathogens to control or suppress the population of a problem weed. Irrespective of the terminology, the approach is a relatively new weed control approach and involves the application of spore suspensions of a weed pathogen in a manner analogous to chemical herbicide applications (Templeton 1982). The use of the inundative approach is based on the fundamental principles of epidemiology. Plant disease is often suppressed by pathogen, environment, or plant factors. Pathogen factors such as low inoculum levels, weakly virulent pathogens, and poor spore dispersal mechanisms; environmental factors such as unfavorable moisture and/or temperature conditions; and plant factors such as low susceptibility of the host, host defense mechanisms, and widely dispersed host populations often limit disease. The inundative approach is an attempt to bypass many of these restraints on disease development by periodically dispersing an abundant supply of virulent inoculum uniformly onto a susceptible weed population. The application is timed to take advantage of favorable environmental conditions and/or the most susceptible stage of the weed's growth. Similarly the spore suspension of the pathogen may be formulated to avoid unfavorable environmental conditions and to facilitate application. As a consequence, the development of an effective augmentation program requires a comprehensive understanding of the pathogen(s) involved, the biology and population dynamics of the target weed(s), the optimum requirements for disease initiation and development, and the complex interactions within the host-pathogen system.

The development of a biological herbicide involves three major phases or stages: 1) discovery, 2) development, and 3) deployment (Templeton 1982). The discovery phase involves the collection of diseased plant material, the isolation of causal organisms, the demonstration of Koch's postulates, the identification of the pathogen(s), the culture of the pathogen(s) on artificial media, and the maintenance of the pathogen cultures in short-term and long-term storage. The development phase includes: 1) determination of optimum conditions for spore production, 2) determination of optimum conditions for disease development and host damage, 3) examination of the infection process, 4) determination of the mode of action of weed pathogens and/or toxins, 5) determination of host range, and 6) quantification of the efficacy of the bioherbicide as a control option. Within the developed world the third phase, deployment, involves close collaboration between researchers, farmers, and the industrial sector for the production, possible commercialization, and use of bioherbicides. Formulation, fermentation, regulatory aspects, marketing, and implementation are essential aspects of this phase. Within the developing world, on-farm production of bioherbicide pathogens may be feasible. Opportunities also exist for small "cottage" industries to develop and produce spores at the village level, as well as large industries to produce bioherbicide products for larger markets throughout the region (Watson 1991a).

Biological control organisms are most often host specific and usually will control only one weed species. As a result, the biological control strategy is narrow spectrum and normally will be used in combination with other weed control methods including hand weeding, mechanical weeding, or chemical herbicides at low application rates, to obtain the broad spectrum control of common weed species complexes associated with rice production systems.

PROGRESS

The primary focus of the biological weed control efforts in Southeast Asia has been on two aquatic weeds, waterhyacinth [*Eichhornia crassipes*

(Mart.) Solms] and water fern (*Salvinia molesta* D.S. Mitchell) (Julien 1992a, Watson 1991a). Recently initiated research on biological weed control has concentrated on three weeds which do not generally infest rice, *Chromolaena odorata* (L.) R. King & H. Robinson, *Mikania micrantha* Kunth, and *Mimosa pigra* L. (Julien 1992a). Most of the previous and current research involves the use of insects, with only a cursory examination of plant pathogens as potential biocontrol agents of tropical weeds.

Two fungal plant pathogens have been registered in the United States, and one has been registered in Canada as a bioherbicide product. DeVine™, a liquid formulation of *Phytophtora palmivora* (Butler) Butler was registered in 1981 for control of strangler vine [*Morrenia odorata* (H. & A.) Lindl.] in Florida's citrus groves. COLLEGO™, a dry powder formulation of *Colletotrichum gloeosporioides* (Penz.) Sacc. f.sp. *aeschynomene* was registered in 1982 for the control of northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.] in rice and soybeans in Arkansas, Louisiana, and Mississippi. BioMal™, a dry formulation of *Colletotrichum gloeosporioides* f.sp. *malvae*, was recently registered in Canada for the control of round-leaved mallow (*Malva pusilla* Sm.) in flax and lentils (Makowski and Mortensen 1992). Another product, LUBAO 2 (*Colletotrichum gloeosporioides* f.sp. *cuscutae*), is being used in China for the control of dodder (*Cuscuta* sp.) in soybean. No other products are as yet registered for use, but active research programs in various laboratories within North America, Europe, east Asia (Japan and Korea), and elsewhere are making rapid progress towards the development of additional products for specific weed problems. Charudattan (1991) provides a recent, comprehensive overview of bioherbicide research.

The inundative or augmentation approach in the tropics is in its infancy but prospects are very encouraging (Evans 1987, Watson 1991a). Some initial surveys, limited collection of diseased weeds, limited isolation of causal organisms, and a few laboratory and field studies characterize the present status of bioherbicide research in the tropics. For example, numerous fungi have been isolated and/or reported on waterhyacinth, but the potential of these fungi has not yet been thoroughly investigated in Asia. Evans (1987) has provided a comprehensive review of fungal pathogens associated with eighteen subtropical and tropical weeds, and concluded that prospects for utilizing pathogens to control half of these weeds are very good.

One of the target weeds of Evans' (1987) review was itchgrass [*Rottboellia cochinchinensis* (Lour.) W.D. Clayton], an upland weed which has been a target of the International Institute of Biological Control (IIBC). Various pathogens were isolated from diseased *R. cochinchinensis*, but most were also pathogenic on maize. However, studies are continuing with two fungal isolates, a *Colletotrichum* species and a *Sphacelotheca* species.

Recently, a biological weed control research program has been initiated at the International Rice Research Institute (IRRI) and the University of the Philippines (UPLB), Los Baños, Laguna, Philippines, in collaboration with McGill University, Montreal, Canada, to evaluate the prospects of utilizing indigenous fungal plant pathogens for the control of major weeds in rice (Bayot *et al.* 1992, Watson 1991a). The primary target weeds are *Cyperus difformis* L., *C. iria* L., *C. rotundus* L., *Echinochloa colona* (L.) Link, *E. crusgalli* (L.) P. Beauv., *Fimbristylis miliacea* (L.) vahl, *Mimosa invisa* Mart., *Monochoria vaginalis* (Burm.f.) Kunth, and *Sphenoclea zeylanica* Gaertn. Virulent pathogens have been isolated from *C. iria*, *E. colona, M. invisa*, and *S. zeylanica*. The latter two weed species have been controlled (100% mortality) in recent field trials at IRRI, and work is continuing with these and other pathogens associated with these target weeds. This work is summarized by Bayot *et al.* in their paper presented at this conference*.

In Japan and in neighboring countries, research is progressing on *Eleocharis, Echinochloa*, and other target weeds (Imaizumi *et al.* 1991, Suzuki 1991, Yoo 1991). Considerable research effort is being directed towards isolating active phytotoxic metabolites from weed fungi and other microorganisms (Yamaguchi personal communication, Yoo 1991).

OVERCOMING THE RESTRAINTS

Pathogen virulence and fastidious environmental requirements are the two restraints to bioherbicide development most often cited (Charudattan 1991, Templeton 1982, Watson 1989). Many aspects of the bioherbicide pathogen such as increased virulence, improved toxin production, altered host range, resistance to crop production chemicals, altered survival or persistence in the environment, broader environmental tolerance, increased progagule production in fermentation systems, enhanced tolerance to formulation process, and innovative formulation approaches are targets for genetic and other biotechnological improvements of bioherbicide pathogens (Templeton and Heiny 1989). Although many bioherbicide pathogens do not produce phytotoxins, or at least are not known to, many microorganisms produce phytotoxins that are highly selective and efficacious at low concentrations and may provide leads to the development of novel weed control products.

Recombinant DNA techniques are well developed for prokaryotes, but few bacteria are being investigated as prospective bioherbicides. Techniques are less well developed for fungi, but significant advances in the ability to manipulate fungal pathogens genetically have occurred in the past 3-5 years (Kistler 1991). Mutation, recombination, and direct gene transfer are methodologies available to genetically modify fungi. Transformation, site directed integration, and gene replacement manipulation have all been accomplished in fungal plant pathogens (Kistler 1991). Only limited efforts to genetically modify bioherbicide fungal pathogens have occurred, but the information and techniques developed and applied to crop pathogens can also be applied to bioherbicide pathogens. The major impediment to the application of recombinant DNA technology to bioherbicide development is the lack of fundamental knowledge of fungal pathogenesis. The limited information available is restricted to crop pathogens, and may not be applicable to bioherbicide pathogens. The mechanisms of pathogenicity are likely to be different in different genera of fungi, and even if efficient transformation systems were commonplace, few desirable "genes" are presently characterized and available for insertion into a bioherbicide pathogen (Kistler 1991). The absence of clear regulatory policies in many countries on the development and use of genetic engineering is also a major impediment to the application of biotechnological applications to bioherbicides. It must also be pointed out that standard strain selection offers excellent opportunities to enhance desirable traits of a candidate weed pathogen.

Optimization of spore production ("fermentation") is often a critical aspect in determining the success or failure of a bioherbicide prospect (Boyette *et al.* 1991, Stowell 1991). Some bioherbicide candidate pathogens do not readily sporulate in culture, and various chemical engineering techniques must be employed to overcome these limitations. Optimization of nutrients in the fermentation medium, the culture environment, and economic aspects are critical to successful bioherbicide development (Stowell 1991).

In addition to pathogen virulence, the need to provide a bioherbicide product that has an acceptable shelf-life and that will survive a degree of adverse environmental conditions has also been a major restraint to bioherbicide development. Advancements have been made in the formulation and application of bioherbicide products, including the use of alginate gel technology, microencapsulation, invert emulsions, and various additives to enhance germination, virulence, and efficacy (Boyette *et al.* 1991, Connick *et al.* 1991). Formulation of bioherbicide products, however, remains a primary area for improvement.

THE FUTURE

Prospects for the development and utilization of bioherbicide technology for major rice weeds are very good. Work in this area is preliminary for the most part, but virulent pathogens of some potential weed targets have been identified and initial laboratory and field results are encouraging. Increased activity in basic and applied science and in biotechnology have a definite role to play in development, implementation, and advancement of this weed control strategy in tropical and subtropical regions. Virulence, efficacy, fermentation, formulation, and application are aspects of prime importance. Industry must become more involved in small niche markets, and techniques must be developed for subsistence farmers as well as modern ones. There is likely to be increased pressure from public and governmental bodies to reduce the use of chemical herbicides. We are challenged to find acceptable, effective complementary weed control tactics.

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DISCUSSION

Dr. Waterhouse as an entomologist was impressed by the promise of bioherbicides, and drew attention to classical biocontrol using fungi. He referred to three examples from Australia where introduced weeds were being effectively controlled by fungi introduced and liberated after careful testing. One is a fungal pathogen, *Puccinia chondrillina*, of the weed *Chondrilla juncea*, which occurs in wheat fields. This pathogen had spread over most of eastern Australia in the period of nine months after it was liberated, and is estimated to be saving the wheat industry $10-20 million per year, without any need for extra inputs. Another example is the rust *Phragmidium violaceum* on blackberry, and a third example is a fungal pathogen on the burr, *Xanthium pungens.*

He suggested that, once exotic weeds have been introduced accidentally onto rice or other crops in Southeast Asia, there is a possibility of introducing fungi which are not already present to control them, provided it is safe to do so. Dr. Waterhouse proposed that one weed which might be controlled in this way is *Rottboellia cochinchinensis*, and said that it might be worth considering the introduction of highly specific fungi to control it.

Dr. Watson mentioned that his own initial research work had been on the possible introduction of rust pathogens into North America as weed control agents. With regard to control of *Rottboellia cochinchinensis* by rusts, he referred to the work of Harry Evans and pointed out that some of these rust pathogens are potentially pathogenic to maize. He felt that the basic problem is the fear of undesirable consequences from introduction, and that many countries in Southeast Asia might be unwilling to agree to the introduction of fungal pathogens, even though these had been demonstrated to be highly specific. Asian scientists might well find that they had identified good pathogens but were unable to get permission to introduce and establish them.

Dr. Moody was interested in the possibility of changes in the virulence of the pathogen applied to weeds. He pointed out that a strain of pathogen present on a particular plant tends to change over time. A particular selection process selected the pathogen initially, but over time the weed may become tolerant and the pathogen adapt to these changes in the plant. Dr. Watson felt that since the most appropriate pathogen strain is selected initially this is unlikely to change, but agreed that if a proportion of the weed population is tolerant, then continued use might select for a weed population which is increasingly tolerant of the pathogen. He suggested that in a system which uses locally produced inoculum rather than a commercial pathogen, there should be an effort to try continually to select for improved strains, so that the next generation might maintain its virulence against the current weed population. Weed control for subsistence farmers might in fact represent a system which is more conducive to maintaining high virulence, because farmers continue to take their samples from the field each year in order to reproduce the pathogen for use the following year.